CEREBRAL VASCULAR STAINING:

A study on goat heads

by

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INTRODUCTION

The introduction of the surgical microscope and high quality imaging techniques demands a higher level of anatomic knowledge from Neurosurgeons. In response to this need, the use of cadaver specimens for anatomic study is undergoing a revival. Cadaver dissection remains the best available method, outside the operation theatre, to understand human anatomic features.

The utility of neurosurgical cadaveric dissections can be improved by defining its vascular anatomy. These vascular anatomic features, which are integral to neurosurgical procedures, are much more clearly defined in injected specimens.

The current methods of staining the cerebral blood vessels are expensive and the materials are not easily available. To fulfill the need to stain the cerebral vasculature, it was decided to develop an inexpensive method using easily available materials, to study the brain as a dissection specimen and as an anatomical model.

This is a **descriptive study**. It was conducted on goat heads in which a number of staining materials and suspending media have been experimented upon to determine their efficacy in staining cerebral blood vessels, choosing materials that are easily available and not expensive.

This study is presented as a long process of trial and error during the course of experimentation, the lessons learnt, the development of the technique, and the final outcome when a suitable colouring agent and suspending medium was selected which satisfied the aim of the study.

AIM AND OBJECTIVES

Aim –

To develop a suitable methodology and to standardize a staining agent for staining the cerebral vasculature.

Objectives –

- To determine the efficacy of different paints and suspending agents in adequately staining the cerebral blood vessels of a goat.

- To ensure that the materials used in the study are readily available and that the methodology is cost effective.

LITERATURE REVIEW

Early knowledge of vascular anatomy was first obtained from the dissections of anatomists including Herophilus (circa 340 BC), who coined the term “artery”.[1] Galen (circa 129 to 200 AD), who established the basis of modern anatomy after his animal dissections; and Vesalius (1514 to 1564), who performed extensive human dissections.

The discovery of capillaries was made independently by Marchello Malpighi (1628 to 1694) and Antoni van Leeuwenhoek (1632 to 1723) in 1661 and 1668, respectively.[1]

Sir Thomas Willis (1621–1675) was a very successful practising physician and an accomplished organizer, teacher, and researcher. He performed necropsies on his patients and did extensive anatomic dissections, especially on the brain. He was probably the first to study the circulatory supply of the brain in detail. He wrote the following about the anatomy of the vertebral circulation :

as the Carotides carry the tribute of the blood to the brain; so the Vertebrals serve chiefly for watering the cerebellum and the hinder part of the oblong marrow. . . . The Vertebral Artery passes through little holes cut in the extuberances of the Vertebrae till it comes near the base of the skull and is admitted through the last hole. . . . Beneath the Cerebellum the

Vertebral branches are united.[2]

Willis is usually remembered as the describer of the vascular composition of the large arteries at the base of the brain, the so-called circle of Willis. He emphasized the capability for collateral circulation if an artery became blocked and the interconnection of blood vessels. A section in his anatomy text is devoted to "for what use the wonderful net is made, and the reason for it."[2]

In 1889, Manchot published his classic work – *Die Hautarterien des Menschlichen Korpers*, detailing the cutaneous circulation of the whole body.

Although it is a relatively recent advance, the use of coloured injections into the vascular tree is an old art. The first injection techniques relied on coloured materials which were liquid when warm and solidified upon cooling, such as wax mixed with paint. These techniques, called *warm injection*, were used with great success as early as the 17th century. By the early 19th century, *cold injection*, a technique that relied on a catalyst to harden a coloured liquid had been established.[3]

Non-radiopaque Injection Studies

Jean Riolan (1580 to 1657) was the first anatomist to inject colored dyes to demonstrate the branching of the vascular tree.[1] Whitten[4] summarized the various dyes used historically in the study of vascular anatomy. These included saffron, carmine, Prussian blue, India ink, and silver nitrate in aqueous or gelatin suspension. India ink injections have since been extensively used for the study of vascular territories to plan surgical flaps.[5] Ink can be used to stain blood vessels to facilitate their dissection. Gelatin can be mixed with the ink to facilitate dissection.

Injection Masses

Various “injection masses” have also been used to fill blood vessels to facilitate dissection. Swammerdam (1637 to 1680) injected colored wax in arteries and veins. He then dissected the specimen and covered it with resin.[6] Substances such as starch, plaster of Paris, glue, glazier’s putty, asphalt, latex or rubber, gum Arabic, sodium silicate, oil of sesame, shellac, thymol, and mercury were injected for this purpose.[4] Latex is still widely used as a visual guide during gross dissections[6] and as a suspending medium for various radio contrast media. Its color makes it easier to identify vessels during dissection and its elasticity helps preserve vessel integrity. Latex is supplied in a liquid form and solidifies quickly in

the presence of formalin. The standard injection technique takes advantage of this property. After flushing out blood, latex is injected in the arterial system and is allowed to distribute for a few minutes. Formalin is then injected in the venous system and slowly diffuses throughout the tissues and microvasculature. This hastens the hardening process of the latex necessary for dissection. Another, less reliable method consists of first injecting the formalin intra-arterially and then injecting the latex.

Diaphanization (Spalteholz Method and Derivatives)

This process involves rendering cadaveric tissues transparent by a series of chemical reactions according to a method developed by Spalteholz.[7] The basic steps consist of (1) injection of India ink or latex to allow later visualization of vessels, (2) fixation of the specimen with formalin, (3) optional decalcification, (4) bleaching, (5) dehydration with alcohol, and (6) rendering the specimen transparent with a combination of methyl salicylate and other products. Diaphanization has been mainly used for studying the vascular anatomy of bone, tendons, and joints. It has also been used for study of surgical procedures. This method allows visualization of vascularization in situ with proper intravascular staining. However, the procedure is time consuming and can only be applied to relatively small and thin specimens.

Tissue Corrosion

Tissue injection followed by corrosion has also been used extensively to define vascular anatomy. Most techniques involve the use of a medium with a low melting point that is injected at a higher temperature and allowed to solidify.[6] Bidloo (1685) used Rose's metal (alloy of bismuth, lead, and tin) as an injectant and boiled the specimen to remove connective tissues. Ruysch (1704) injected metal in coronary vessels and used maggots and larvae to decompose the tissues and obtain a vascular cast. Lieberkuhn (1711 to 1746) was the first to use acid to corrode tissues. Since then, various mixtures of injectants have been used, and tissues surrounding the casts have been corroded with various agents such as hydrochloric acid or potassium hydroxide.[4] Corrosion casts are still in use today to study surgical anatomy. Various resins have been used: epoxies, methymethacrylate,[8] and acrylic. Some of them, such as acrylic, do not penetrate the capillary bed of human organs.[9]

Radiopaque Injection Techniques

In 1895, Roentgen discovered x-rays, and within a few weeks of this discovery, the first angiogram was produced by Haschek[10] in 1896 after injecting chalk into the arteries of a cadaveric hand. Other early contrast media include: [4,11]

- calcium sulphate,
- mercury,
- barium, [12]
- bismuth,
- colloidal silver,
- silver iodide,
- lead oxide,
- lead chromate,[13]
- lead phosphate,
- white lead,
- vermilion (mercuric sulfide),[11]
- strontium bromide
- sodium bromide,[14] and
- iodized oils.

The most useful of these methods have been barium sulphate and lead oxide injection techniques.

Barium Sulphate

Barium sulphate is a well-known radiographic contrast agent. Its use was first described in 1920.[4,12] The technique involves flushing out intravascular blood and injection of barium sulphate mixed with gelatin or latex to facilitate subsequent dissection. It has been used with moderate

success in the early investigation of cutaneous vascular anatomy. Barium was soon replaced by lead oxide as the standard contrast agent for the study of very fine vascular networks such as those found in the integument.[15] However, the barium sulphate technique has been reappraised lately. Some authors have improved the technique and obtained angiograms of very high quality.[16]

Lead Oxides

Lead oxide is the standard for visualization of blood vessels required for the planning of surgical flaps. It is not expensive, is simple to prepare, and reliable results are obtained. Lead oxide in gelatin was first used by Jamin and Merkel in 1907.[17] Salmon, in 1936, perfected the technique to study muscle and skin vascular anatomy by injecting red lead. In 1986, Rees and Taylor rediscovered Salmon's work and proposed their simplified lead-oxide injection technique.[15] They used lead oxide in its litharge form (PbO) instead of red lead (Pb_3O_4), and gelatin was re-introduced in the mixture to facilitate dissection. This combination is highly radiopaque; it perfuses the small radicles of the vascular tree and sets to a firm rubbery consistency to facilitate dissection. However, Taylor has gone back to Pb_3O_4.[5,18,19] The bright orange-red color of the lead is very easy to identify and facilitates dissection of fine structures. Different forms of lead oxide used for injection studies are often confused. Red lead,

litharge (Tetragonal PbO), and massicot (Orthorhombic PbO) are degradation products of plattnerite (PbO_2). Red lead oxide is the most frequently used form of lead oxide for injection studies.

Heymans et al[20] compared the techniques of cadaveric injection combining gelatin, lead oxide, and various dyes. The addition of lead oxide did not seem to limit the progression of the mixture into the vascular network, since infra-millimetric arteries displayed a constant and consistent opacity. Unfortunately, when vascular structures overlapped the bony structures, the use of lead oxide did not show the thinnest vessels properly.

In order to enhance the contrast and to keep the opaque properties, rhodamine B was added to obtain a pink coloration. This technique enabled further dissection, in order to study special anatomic relationships demonstrated by prior x-ray examination.

Coloured silicone injection

Silicones or polysiloxanes, are inorganic-organic polymers with the chemical formula $[R_2SiO]_n$, where R = organic groups such as methyl, ethyl, and phenyl. These materials consist of an inorganic silicon-oxygen

backbone (...-Si-O-Si-O-Si-O-...) with organic side groups attached to the silicon atoms. In some cases organic side groups can be used to link two or more of these -Si-O- backbones together. By varying the -Si-O- chain lengths, side groups, and cross-linking, silicones can be synthesized with a wide variety of properties and compositions. They can vary in consistency from liquid to gel to rubber to hard plastic. The most common type is linear polydimethylsiloxane or PDMS. The second largest group of silicone materials is based on silicone resins. The different forms of silicone include fluids, emulsions, lubricants, resins, or elastomers (rubbers). Silicones are highly valued materials because they have a combination of physical properties not found in other polymers. They have outstanding heat stability and can be used in applications where organic materials would melt or decompose.

Coloured silicone injections are used in the major cadaver dissection laboratories around the globe for vascular staining. This is an example of a cold injection, as opposed to the warm injection technique that is reported in this manuscript.

For each adult human head[3], approximately 150 ml is required for the venous tree and 100 ml for the arterial tree. The powdered colour and the thinner are mixed first. The container is closed with a cap and the solutions mixed well. The product is a vividly coloured liquid which is

Table 1 - Preparation of coloured silicone mixtures[3]

Silicone mixture	Sample preparation for one head
Blue	
Blue powder	5 heaping teaspoons
Thinner (1 part)	50 ml
Silicone (2 parts)	100 ml
Catalyst (1:10 with silicone)	10 ml
Red	
Red powder	5 heaping teaspoons
Thinner (1 part)	50 ml
Silicone (1 part)	50 ml
Catalyst (1:10 with silicone)	5 ml

then transferred into a beaker or a shallow jar. The silicone is added till the colour is uniform. Finally, the catalyst is added. The catalyst needs 5 -12 hours to cure, so the injection can be performed unhurriedly. The final red

silicone mixture should be less viscous than the blue one since the arterial tree offers more resistance than the venous tree.

The specimen size determines the amount of solution to be prepared

Since specimen sizes vary, mixtures must be adjusted to provide sufficient quantities for each injection.

Vascular resistance to injection determines the thinner/ silicone ratio

Differences in vessel size should determine the ratio of thinner/ silicone ratio. In cases of greater resistance, the thinner/ silicone ratio can be increased. The condition of the venous and arterial systems can be determined during the injection. The presence or absence of a significant uncontrollable leak and the level of resistance determines the thinner/ silicone ratio.

The amount of pigment (powdered colour) is determined by the thinner/ silicone ratio

The pigment should be added to the thinner solution before silicone is added. The pigment does not readily mix with the more viscous silicone and requires more stirring before entering into the solution. The silicone often bleaches out the mixture, necessitating the addition of more pigment to the solution. It is better to add sufficient amount of pigment before the addition of silicone, to counteract this effect.

Agar agar

The word agar comes from the Malay word "agar-agar" meaning jelly. It was in 1881 that Robert Koch showed the value of agar in culturing bacteria, and imported it from Japan, which had a monopoly on the agar trade.

Agarose (or agar) is a galactose polymer obtained from cell walls of several species of red algae, or seaweed (Sphaerococcus euchema) chiefly from the Ceylon, or Jaffna, moss (Gracilaria lichenoides) and species of Gelidium, chiefly from eastern Asia, Chile and California. It is also known as Kanten, Agar-Agar, or Agal-Agal (Ceylon Agar).

Chemically, agar is a polymer made up of subunits of the sugar galactose; it is a component of the algae's cell walls. Dissolved in boiling water and cooled, agar becomes gelatinous; its chief uses are as a culture medium for microbiological work (e.g. agar plates) and as a laxative, but it serves also as a thickening for soups and sauces, a vegetarian gelatin substitute, in jellies and ice cream, in cosmetics, for clarifying beverages, and Japanese desserts such as anmitsu, as a clarifying agent in brewing, and for paper sizing fabrics.

Agar is generally resistant to shear forces; however, different agars may have different gel strengths or degrees of stiffness.

Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller quantities (0.05-0.5%) are used in media for motility studies (0.5% w/v) and for growth of anaerobes (0.1%) and microaerophiles.

The problem with using gelatin as the base is that many microorganisms release proteases which will digest the gelatin (it's protein-based), causing it to liquify. Agar is preferentially used because it is a carbohydrate derivative and thus unaffected by proteinases. It will retain its gelled characteristic while gelatin turns to soup.

Hysteresis

Hysteresis describes the phenomenon of the differing liquid-solid state transition temperatures that agar exhibits. Agar melts at 85 °C and solidifies from 32-40 °C.

Hysteresis is a property of systems (usually physical systems) that do not instantly follow the forces applied to them, but react slowly, or do not return completely to their original state: that is, systems whose states

depend on their immediate history. For instance, if one pushes on a piece of putty it will assume a new shape, and when one remove one's hand it will not return to its original shape, or at least not immediately and not entirely.

Hysteresis manifests itself in state transitions when melting temperature and freezing temperature do not agree. For example, agar melts at 85 °C and solidifies from 32 to 40 °C. This is to say that once agar is melted at 85 degrees, it retains a liquid state until cooled to 40 degrees Celsius. Therefore, from the temperatures of 40 to 85 degrees Celsius, agar can be either solid or liquid, depending on which state it was before.

Storage of specimens

The use of formaldehyde for storage of specimens has been a standard practice. It provides good long term structural preservation, denatures organisms (including HIV) and prevents microbial growth.[3] The major disadvantage of formaldehyde, in addition to its smell, is the resultant extreme hardening of the brain. There may be health risks associated with prolonged contact or inhalation of formaldehyde. A variety of alternative preservatives have been found to be useful including 66% ethyl alcohol, phenol, polyethylene glycol and phenoxyethanol.

Softening of specimens

The alternatives to formaldehyde fixation that are currently available to make the tissues soft and more life-like either are time-consuming or shorten the period of time for which cadavers can be stored. The effect of fabric softener (methyl bis [tallow amido ethyl] 2-hydroxyethyl ammonium methyl sulfate) on formaldehyde-fixed brains to make them soft and life-like, was tested by Krishnamoorty and Powers.[21] The deformability was measured by using a Schiotz ocular tonometer. They found that the use of fabric softener makes the brain significantly softer ($P < 0.005$) when compared with a standard diluted formalin solution. They also used fabric softener in neurosurgical prosections and found that the brain can be made soft enough to be able to retract. In addition, it can be used to better demonstrate the planes of tissue cleavage, making it very useful in simulating a life-like situation.

Sanan et al[3] ,however, were of the opinion that there was no difference in the softness of the specimen if it is stored in 66% ethyl alcohol as compared to a brain immersed in formaldehyde and then treated with fabric softeners. Moreover, the coloured silicone is completely destroyed after treatment of the specimen with fabric softeners.

Integration of cadaveric dissection with preoperative imaging

Zhao et al[22] standardized a procedure in which they integrated cadaveric dissection with the imaging to understand the pathological anatomy, especially for vascular lesions, to allow greater precision in surgical planning.

Renografin 60 (a CT contrast agent) was mixed with silicone rubber in a ratio of 1:8 and injected into the cerebrovascular systems of 6 cadaveric heads. The blood vessels in CT scans demonstrated a higher attenuation than the surrounding soft structures. Static and dynamic three dimensional images of the vascular tree were obtained as viewed through surgical corridors of various skull base approaches.

This technique allowed both a 3-D CT rendering of the cerebrovascular structures and the cranial base as well as conventional cadaveric dissection. Thus, the surgeon can appreciate the surgical anatomy associated with cerebrovascular lesions and to plan a surgical approach through a virtual 3-D surgical bony opening.

MATERIAL AND METHODS

This study was conducted in the laboratory of the Department of Neurological Sciences, Christian Medical College, Vellore, on decapitated goat heads bought from the local butcher shop. The heads were sectioned at the occipital condyles, due to religious reasons. 14 such heads were stained with different qualities of paints and agents to determine which one was the feasible for cerebral vascular staining.

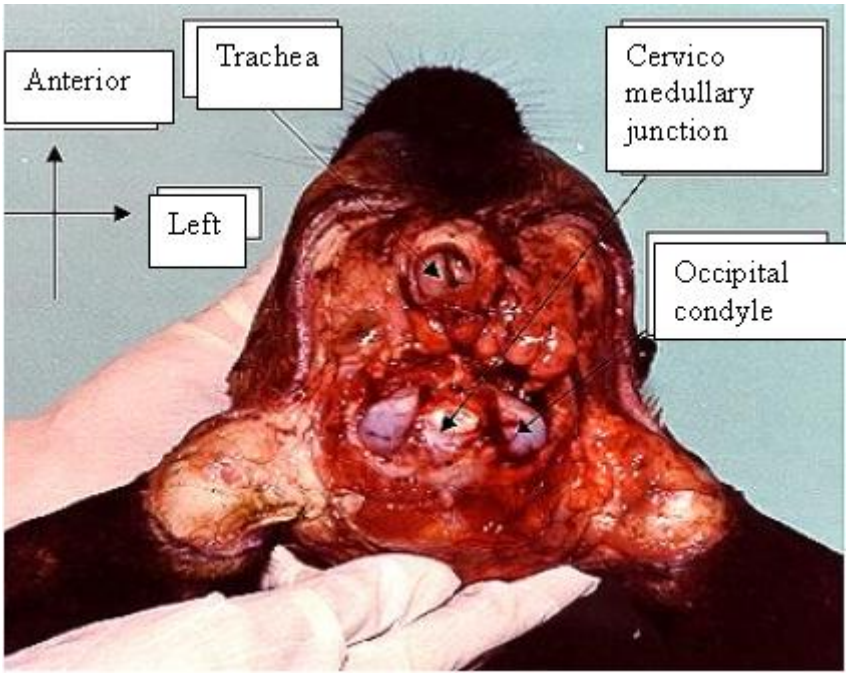

Fig 1. Goat's head sectioned at the level of the occipital condyles

Six steps are required and are summarized as follows:

1) Exposure of the great vessels

The head is stabilized such that the vertex is dependant and the

neck is exposed. A combination of blunt and sharp dissection is used to expose 2-3 cm of the vessel length.

The vessels exposed to inject the colouring agent are:

- common/ internal carotid arteries (2)
- vertebral arteries (2)
- internal jugular veins (2)

The carotid arteries and the jugular veins can usually be located if the head is sectioned at the level of the occipital condyles, however, the vertebral arteries are found only if the sectioning is done slightly lower, at the level of C3 to C4 vertebral body.

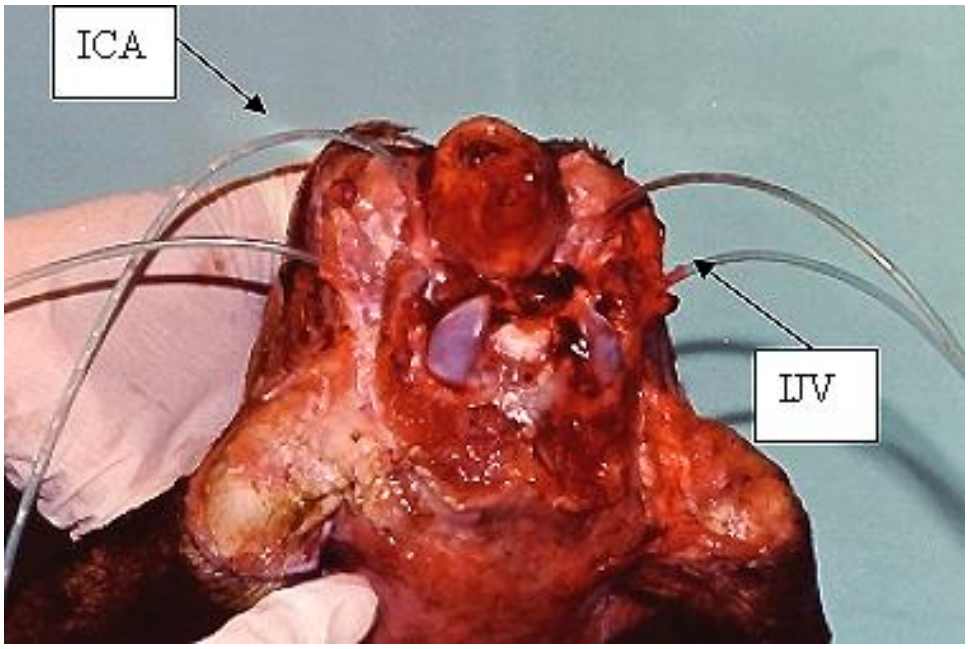

Fig 2. Cannulation of the blood vessels.
(ICA- Internal carotid artery, IJV – Internal Jugular vein)

2) Cannulation of the great vessels

Once the vessels have been dissected free of the surrounding tissues, infant feeding tubes nos. 5 or 6 are inserted into each of them and then secured with sutures (to prevent accidental dislodgement of the cannulae during the injection process). These provide the access for irrigating and then staining the cerebral blood vessels as described below. The two steps above take 45 minutes to an hour to complete.

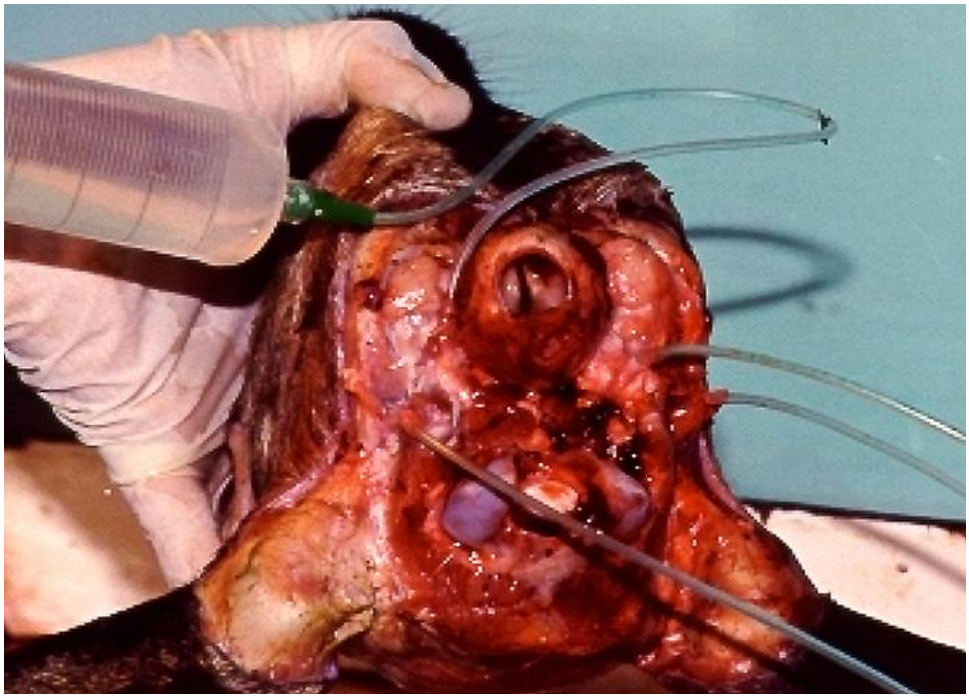

Fig 3. Irrigation of the blood vessels

3) Irrigation of the blood vessels

The goal of this step is to wash the blood vessels free of clotted blood. Failure to perform this step adequately results in a poor vascular injection. Each of the blood vessels are irrigated in turn with water, injected through the infant feeding tubes by a 60 cc disposable syringe.

This process takes up to 30 to 45 minutes and it is ensured that the returns from the partner vessel are clear. Leaking blood vessels are identified and ligated. It is impossible to control every leak and it is acceptable if water oozes from the remaining surface of the neck, however there should not be any individual jets of water. If excess pressure is applied, the cerebellar tonsils are seen herniating out of the foramen magnum. Steps 1 to 3 are done in continuation, in one sitting. The irrigated head is then submerged into 10% formalin solution for 24 to 48 hours. After this, the blood vessels are again irrigated with water to remove the clots which sometimes form (while the brain is submerged in formalin) and to remove any formalin fixative, prior to injecting the staining material.

4) Preparation of the colouring agent

The colouring materials are mixed with the various agents (described later) prior to the injection (red for arteries and blue for veins). The final solution is loaded into a 60 cc syringe for the injection.

5) Injection of the stain

Vessels other than the one to be injected are clamped. Red paint is injected into one of the carotid arteries through the infant feeding tubes till a resistance is felt. At this juncture, the contralateral vessel is opened and the injection is continued till a free flow of mixture flows through the open

Fig 4. Injection of the staining agent into the blood vessels

vessel. As the mixture is draining out of the open vessel, the clamp is replaced and the injection ends. About 15 ml of paint (dilution 1:1 with water) is required to be injected into each internal carotid artery.

Blue paint (dilution 1:1 with water) is injected into the internal jugular veins using the technique described above.

All the blood vessels in the surrounding tissues which are leaking paint are identified and ligated. It is important to ligate the stumps of these blood vessels to maintain adequate forward pressure in the main vessel. In regions where suturing the blood vessels is not possible, the leaking

vessels are sealed with super glue, e.g. the circular sinus around the foramen magnum.

Sometimes the mixture does not emerge from the opposite paired vessel. For the internal jugular injection, this usually represents inadequate obliteration of the open veins over the cut surface of the neck. For arterial injections, this usually indicates a poor quality specimen, with occlusion of the blood vessels.

Steps 4 and 5 take up to an hour to perform. The stained head is again submerged into 10% formalin solution for 24 to 48 hours.

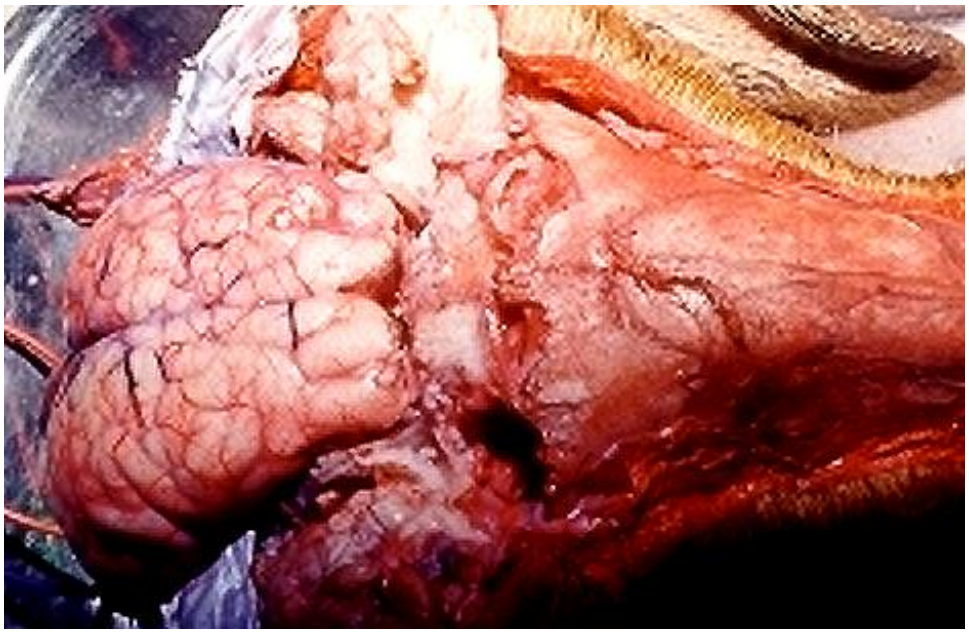

Fig 5.The brain nestled in the skull

6) Evaluation of the final specimen

The quality of the injection can be rapidly evaluated by drilling a burr hole over the parietal convexity. The dura may be opened to reveal brightly coloured cerebral blood vessels. A craniectomy is done after 24 to 48 hours later, to expose the cerebral hemispheres and the posterior fossa contents and the desired anatomy is studied using an operating microscope. Relevant photographs are taken during this session. The basal dura is adherent to the base of the skull and the brain is delivered by severing the cranial nerves which pierce the dura mater to exit the skull. The delivered brain is then kept for further examination.

Staining materials:

a) Poster colours and acrylic paints

In the initial experiments conducted on the goat brains, poster colours and acrylic paints were injected into the great vessels of the neck to stain the cerebral vasculature. The concentration used to dilute the paints with water was 1:1.

A combination of poster colours and acrylic paints was also used with limited success.

b) Paints and suspending media

Since poster colours and acrylic paints could stain blood vessels but could not cast them completely, the next step consisted of premixing the paint with other media in an attempt to harden the paint solution within the blood vessels. The different media mixed with the paints were 5% formaldehyde, egg white, egg white with icing sugar and egg yolk (both heated and unheated). Various concentrations of the paint - medium combinations had to be tested to determine if the paint was solidifying or not. Instead of testing this in the cerebral blood vessels, the mixtures were injected into infant feeding tubes, initially. The infant feeding tubes were cut after 3 and 10 days and it was determined if the mixture within was solidifying.

The mixture which solidified the best within the infant feeding tubes was an egg yolk and paint solution in the ratio 2:1. This was injected into the cerebral vessels.

c) Agar – acrylic paint solution

Lastly, the combination which gave the best results was a boiled solution of acrylic paint and agar agar. Agar has the property of gelatinising after it is boiled and then cooled to 32 to 40 degree Celsius.

This property of agar, termed as hysteresis,* was put to test in this experiment to stain the cerebral blood vessels.

Agar was mixed and heated with poster colours and acrylic paints, in turn to determine which one is a better colouring agent. The agar-acrylic paint mixture became gel-like on cooling, while the poster colour-agar solution remained liquid on cooling.

Agar and water was boiled first before mixing with acrylic paint. The result was an inhomogenous mixture with some of the paint flaking off. Thus it was decided that the entire mixture (agar + water + acrylic paint) must be mixed and then heated to produce a homogenous solution.

The paint and agar were mixed and heated till the boiling point, loaded into a 60 ml syringe and injected into the blood vessels. Usually, heated agar-paint solution takes about 3 to 5 minutes to solidify; hence the injection should not be delayed.

The red paint which was injected into the arteries was diluted to 20% of its concentration as the arterial walls are thicker and have narrower lumen, hence provide resistance to a more viscous solution. The veins, however, have thinner walls and they provide lesser resistance to the

* Refer to page 20

injection. Due to this reason, the blue acrylic paint was diluted to 50% of its original concentration before injecting it into the internal jugular veins.

All the different types of dyes/ staining mixtures were injected into the carotid arteries and the jugular veins as described above. The final evaluation was done by a craniectomy and durotomy, after 24 to 48 hours of the injection, relevant photographs taken and the specimens kept in 10% formalin for further study.

RESULTS

14 goat heads were injected with various stains and colouring agents using the above technique, from September 2003 to November 2004.

Acrylic paints and poster colours

The initial experiments were conducted by injecting poster colours and acrylic paints into the blood vessels – blue for veins and red for arteries, after having irrigated the specimen, twice, with a gap of one day.

The red and blue paints were diluted in equal quantities of water before injecting them into the carotid arteries and the jugular veins respectively.

Figure 6 reveals that there is incomplete staining (fig. A, arrow on the unstained right middle cerebral artery) and patchy uptake (fig. B, arrow on the right posterior communicating artery). This was probably due to improper irrigation technique which was overcome in subsequent dissections. The other possibilities were the presence of clots which could not be evacuated with the irrigation or due to vascular stenosis.

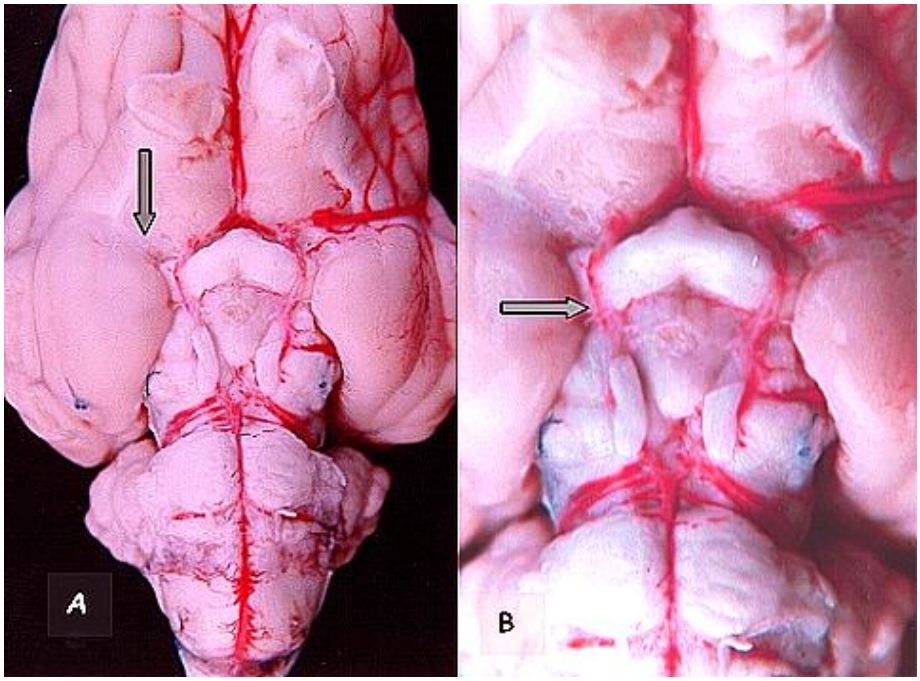

Fig 6. Blood vessels stained with acrylic paints - incomplete staining and patchy uptake of the stain within the blood vessels.

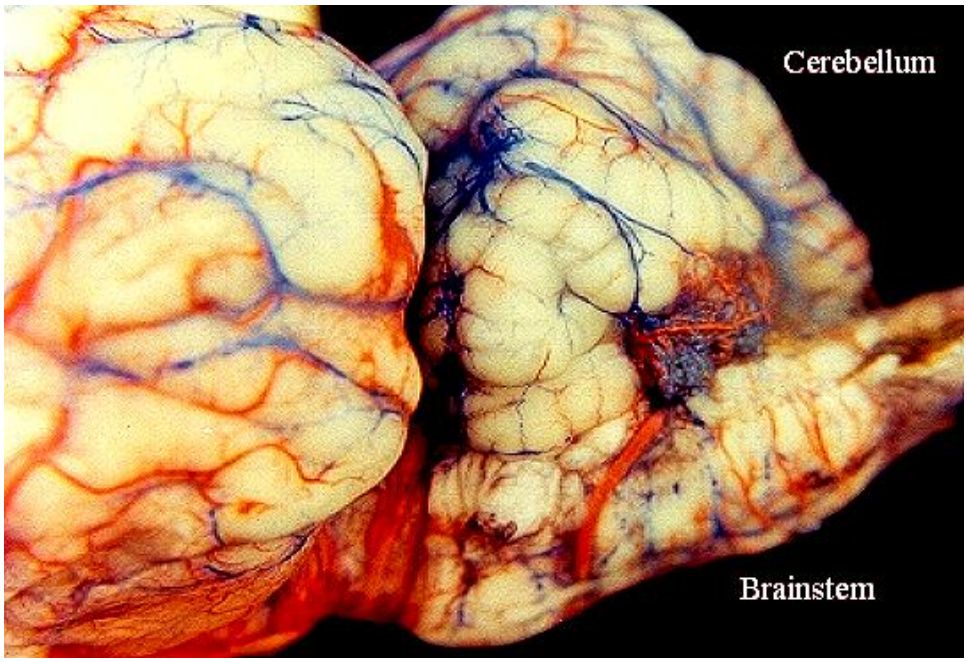

Fig 7.Blood vessels injected with poster colours. Photograph showing the lateral aspect of the cerebellum and the brainstem on the right and the posterior part of the left occipital lobe on the left.

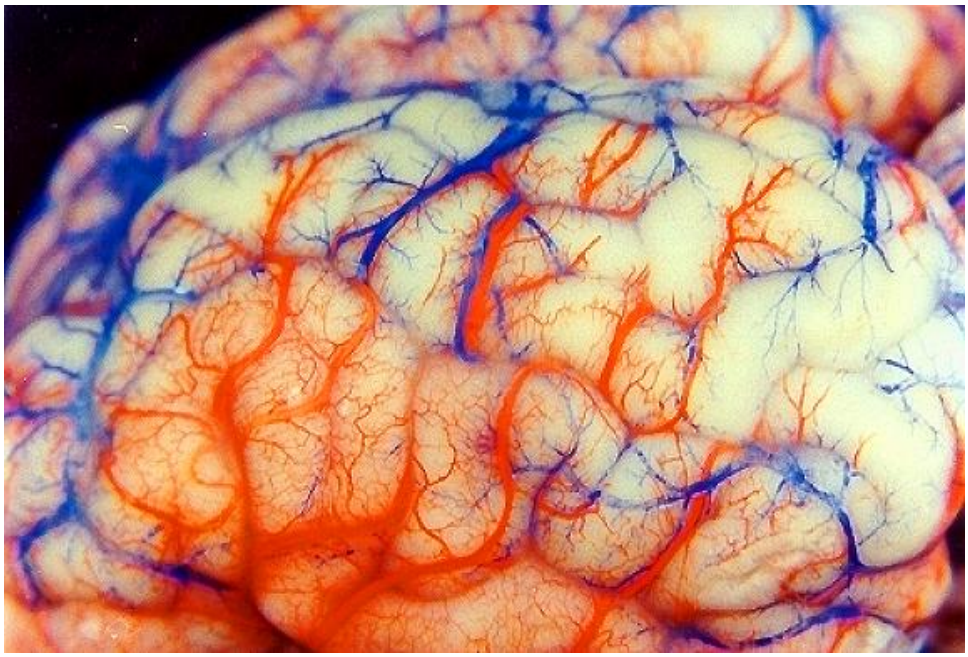

Fig 8. Poster colours. Photograph shows the left cerebral hemisphere (lateral view). The sylvian fissure is seen inferiorly with the branches of the middle cerebral artery climbing up the inferior frontal gyrus.

Fig 9. Poster colours. Photograph showing the superior and inferior surfaces of the brain

Poster colours were diluted with water in a ratio of 1:1 prior to injection. This gave a better result as far as brightness of colour and penetration into the smaller, distal vessels was concerned (Figures 6 to 9). However, the colour in blood vessels tended to fade by a week to ten days.

Although acrylic paints did not stain the blood vessels as brilliantly as the poster colours did and tended to be a bit patchy, had the advantage that they did not fade with time and to a certain degree were able to fill the lumen of the blood vessels.

At this juncture, it was thought that injecting a combination of these two types of paints would overcome the disadvantages that they possessed individually. Poster colours could stain the distal vessels well (unlike the acrylic paints), but tended to fade soon. Acrylic paints, on the other hand could fill up the vessels better than poster colours but could not stain the distal vessels.

After having understood the qualities of the acrylic paints and poster colours, one specimen was injected with poster colours initially followed by another injection of acrylic paint through the same blood vessels. The rationale was that the initial injection of poster colours would fill up the distal vessels and the latter injection with acrylic would help in delaying the fading of colours and will cast the blood vessels.

Theoretically, this was a logical surmise. After the craniectomy, the results were quite disappointing. The distal vessels and the bigger vessels were inadequately stained. This was probably because the latter injection of acrylic paints had washed out the poster colours and the two did not mix well with each other.

Paints and suspending media

Even though the acrylic paints and poster colours were staining the blood vessels, they could not solidify within the blood vessels and fill them up. In an attempt to overcome this problem, acrylic paint was premixed with other suspending media to determine if the vessels could be casted with that particular combination. These suspending media into which the paints were mixed included the following:

- 5% Formalin
- Egg white
- Icing sugar
- Egg yolk

Several such combinations of acrylic paints and the various suspending media (heated and unheated) were injected into infant feeding tubes; results of which are as follows:

Table 2 - Results of acrylic paint – suspending medium mixture injected into infant feeding tubes

Mixture	**Result**
Hot –	
E_1 P_1 W_1	not solidified
E_1 P_1 W_1 S_1	not solidified
Y_1 P_1 W_1	not solidified
Cold –	
E_1 P_2	not solidified; better than the heated mixture
E_1 P_1	not solidified
E_4 P_1 F_1	not solidified
E_4 P_1 F_1 S_5	not solidified
E_5 P_1	not solidified
E_5 P_1 S_5	not solidified
Y_1 P_1	semi-solid
Y_1 P_1 F_1	slightly semi-solid
Y_2 P_1	**semisolid; the best so far**
Y_5 P_1	slightly liquid, as compared to Y_2 P_1

Note: Water – W, Paint – P, 5% formalin – F, Egg white –E, Icing sugar – S, Egg yolk – Y
E_5 P_1 means 5 parts of egg white + 1 part of paint

This experiment showed that heating egg white or yolk does not help in solidification of the mixture. The egg yolk and paint mixture (2:1) was solidifying well in the infant feeding tubes and higher concentrations did not yield better results, hence it was injected into the jugular veins.

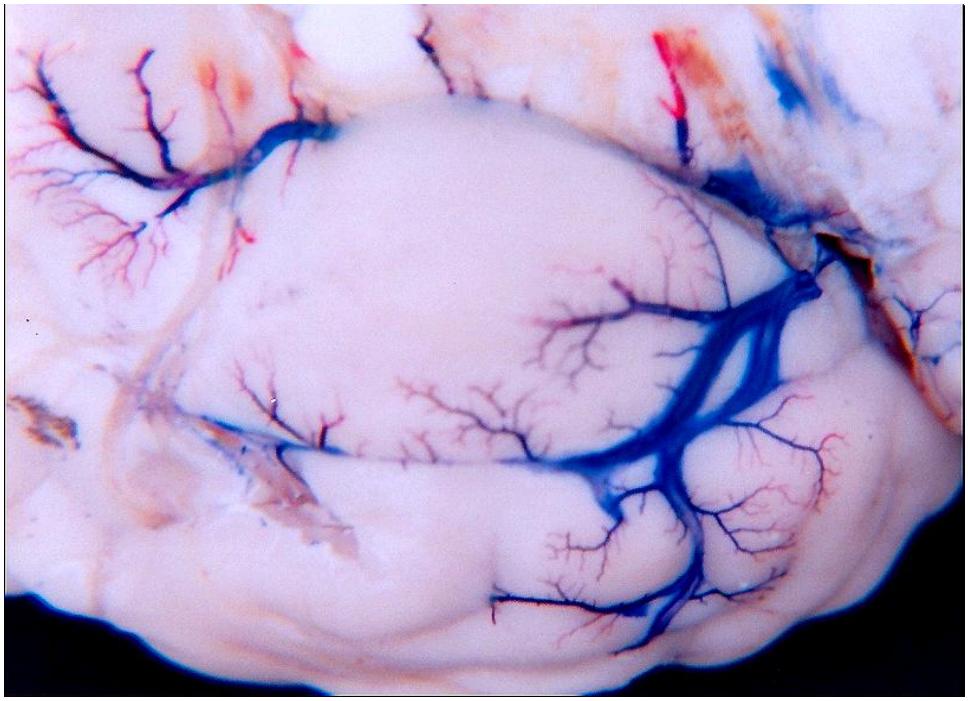

Fig 10. Veins stained with egg yolk and acrylic paint (2:1) mixture

It was observed that the blood vessels were partially filling up with the egg yolk- paint mixture and there was no spillage of the dye into the surrounding structures. This was definite step forward in the direction of staining the blood vessels and making them appear cylindrical, as if filled with blood.

Agar – acrylic paint solution

After the success with the egg yolk and paint solution in staining and filling the blood vessels, the next suspending medium that was experimented upon was agar (Agar-agar).

In order to stain the blood vessels optimally with the dye, the correct concentration of the dye must colour the blood vessels and harden within their lumen so that the blood vessels appear cylindrical. In other words, the dye's concentration must be such that it is not too dilute to leak out of the vessels' lumens thus preventing adequate staining, nor must be too viscous in which case it will not enter the smaller blood vessels. It is this fine balance which must be achieved to stain and cast the blood vessels optimally. The correct concentration of the agar-acrylic paint mixture which would be neither too dilute nor too viscous had to be standardized next.

Four goat heads were experimented upon to standardise the concentration of the dye (agar-acrylic paint mixture). The results of the standardisation process are as follows:

Table 3 - Results of agar – acrylic paint injection

Brain	Arteries/ Veins	Paint : water	% agar	Results
A	arteries	1:2	0.5%	Very few arteries were stained
A	veins	1:2	1%	None of the veins were stained
B	arteries	1:4	1%	Circle of Willis stained partly
B	veins	1:2	1%	Only superior sagittal sinus stained
C	arteries	1:4	3%	Circle of Willis stained well. Smaller arteries at the base of the brain and convexity vessels did not stain well
C	veins	1:1	4%	Poor staining in superior sagittal sinus and its tributaries
D	arteries	1:4	2%	Circle of Willis and arteries on the skull base were well stained. Moderately good staining in the convexity vessels
D	veins	1:1	2%	Superior sagittal sinus and its tributaries well stained. Vessels on skull base stained

In Brain A, a 0.5% agar- paint solution was injected into the arteries and a 1% solution into the veins. After the craniectomy, it was observed that few arteries had been stained, however the veins had not been casted at all. It was inferred that the dye was too dilute and did not cast the blood vessels.

In brain B, a higher concentration of the dye was injected into the arteries – (1% agar-paint solution) and the arterial circle of Willis was semicasted; an improvement over the vessel staining in brain A. A 1% agar-paint solution was injected into the veins (same as in brain A), because it was felt that the veins in brain A had not been adequately irrigated prior to the injection and this concentration of the dye needed to be experimented upon again before making any inferences. Sure enough, the results were better than in the previous specimen. The superior sagittal sinus had been stained and casted with the blue dye. The results seen in the veins on the cerebral convexities and the base of the brain were disappointing. Hence, it was decided to increase the concentration of the agar for injecting the next specimen.

A 3% agar-acrylic paint solution was injected into the internal carotid arteries and a 4% solution into the internal jugular veins in brain C. The circle of Willis was well stained and casted, but the vessels on the base of the brain and on the cerebral convexities had not. In the venous

tree, there was no uptake of the dye. This injection proved that a 3% dye injection into the arteries and a 4% dye injection into the veins was too thick to overcome the vascular resistance.

The next brain, (brain D) which proved to be the last one, was injected with a 2% dye solution in both the arterial and the venous tree. The red paint which was injected into the internal carotid arteries was diluted to 20% of its concentration and the blue acrylic paint was diluted to 50% of its original concentration prior to injection into the internal jugular veins.[*] A craniectomy was done after 24 hours of the injection which showed that the circle of Willis and arteries on the skull base were well stained. The blood vessels on the cerebral convexity were moderately well stained. The superior sagittal sinus and its tributaries had stained well. Veins on skull base had stained moderately well.

After having experimented with different concentrations of agar – acrylic paint solutions, the best result in staining and filling of the cerebral blood vessels was obtained by injecting a **2% agar-acrylic paint solution** into the internal carotid arteries and the internal jugular veins. The figures below (Figs. 11-14) illustrate the staining and filling of the blood vessels with 2% agar – acrylic paint solution whose concentration was standardized, as above.

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[*] Refer to page 32

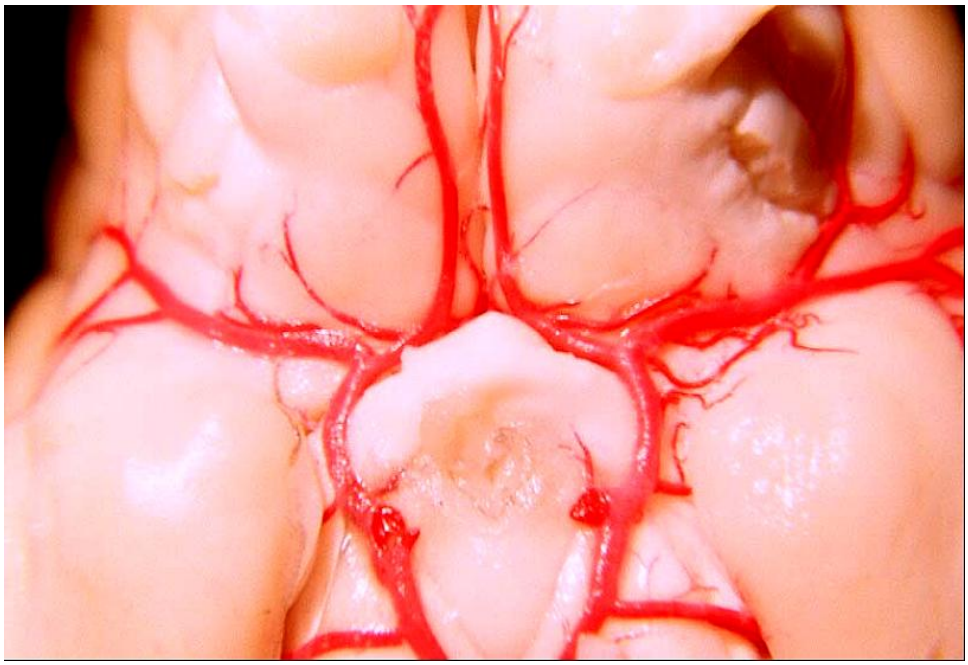

Fig 11. Arterial tree injected with 2% agar-acrylic paint solution showing vessels at the base of the brain stained and casted well

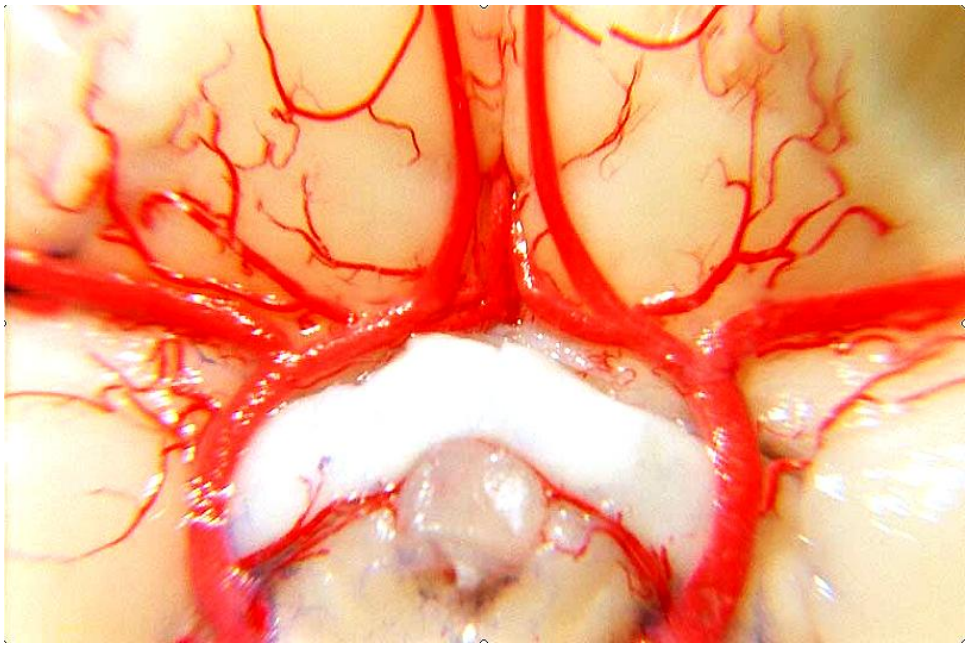

Fig 12. Arterial circle of Willis stained with 2% agar-acrylic paint solution showing how well the blood vessels are filled with the solution after it has solidified within their lumen. Note that there is no spillage/ smudging due to the injection medium.

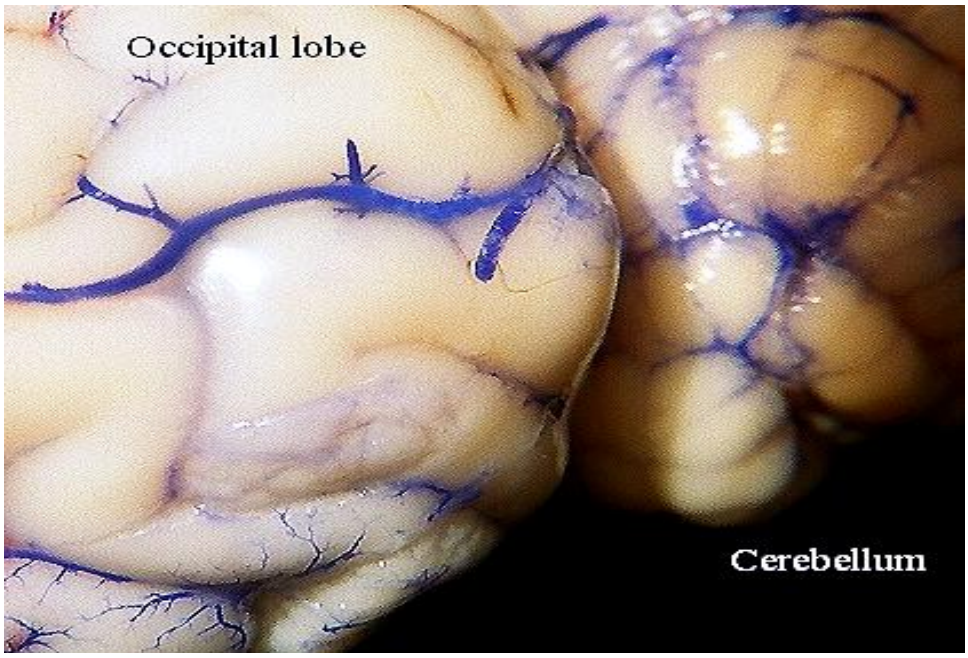

Fig 13. 2% Agar acrylic paint solution. Side view of the brain showing the filling of the veins on the cerebellum and the adjacent occipital lobe

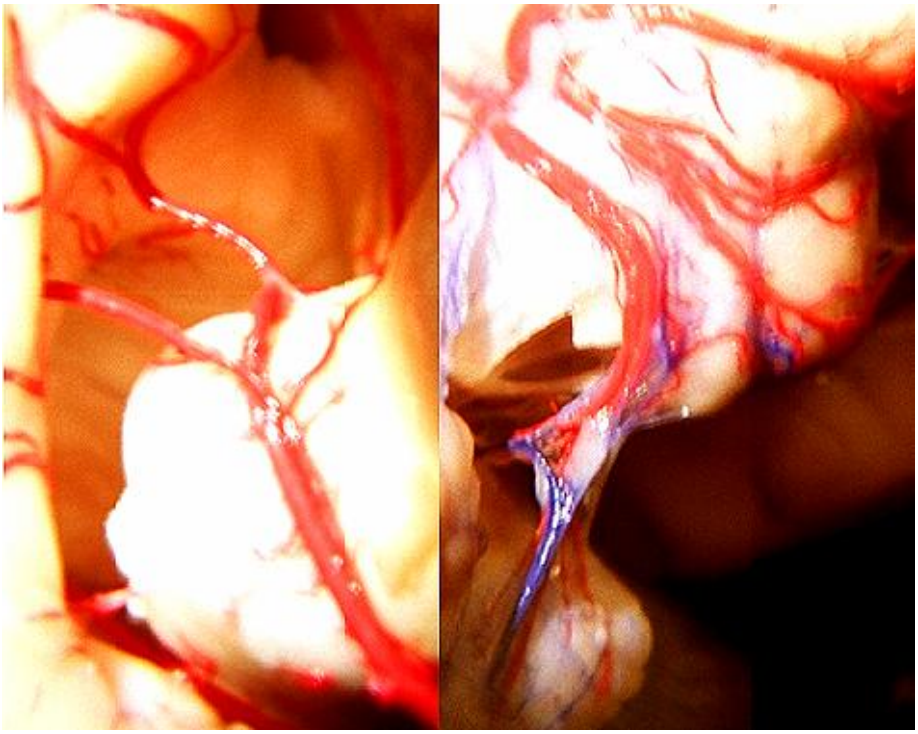

Fig 14. Blood vessels injected with 2% agar-acrylic paint solution. The vessels have been adequately casted and they do not collapse as they cross the cisterns or fissures.

An important determinant of the adequacy of the staining technique and the staining agent is that the portions of the blood vessels which cross cisterns or fissures must stand out rigidly like cords and not collapse, as shown in figure 14.

The above results reveal that the best results in terms of staining and filling of the lumen of the cerebral blood vessels were obtained with a 2% agar – paint solution.

Cost accounting

One of the objectives of this study was that the materials used were readily available and inexpensive. Towards that end, every effort was made to ensure that all the consumables were available locally and within the budgeted amount. A grant was sanctioned by the Fluid Research Committee of Christian Medical College, Vellore, to conduct the study.

Table 4 shows the consumables used to stain one goat head, their quantity and the expenditure involved.

Table 4 - Expenditure incurred (in Indian Rupees) to stain one goat head

Consumables	Calculation	Amount
Acrylic paints	15 x 2 =	30.00
Infant feeding tubes	9.6 x 4 =	38.40
Agar agar	1840/500 x 2 =	07.36
10% formalin	196/2 =	98.00
Total amount		**173.76**

- Two bottles of Acrylic paints (one red and one blue – 15 ml each) cost Rs. 15 each. They were bought from a stationery shop.
- 4 infant feeding tubes (no. 5 for the internal carotid arteries and no. 6 for the internal jugular veins), each costing Rs. 9.60 were bought from the hospital pharmacy.
- 500 g of Agar agar powder (costing Rs 1840) was purchased from a medical store and 2 g was used in one specimen.
- 1 bottle of 40% formalin (costing Rs 196) was purchased from the same medical store and diluted to obtain a 10% solution. Half the diluted volume was required for storing one specimen.

The total cost involved to stain one goat head with 2% agar – acrylic paint solution was Rs. 173.76 (3.70 USD).

DISCUSSION

The dissection of cadaveric specimens is important for a sophisticated understanding of neurosurgical anatomic features and approaches.

The technique

This was a **descriptive study**, in which efforts were made to standardise a dye which had not been used earlier by others in order to stain the cerebral vasculature. Hence, it necessarily involved many a trial and error.

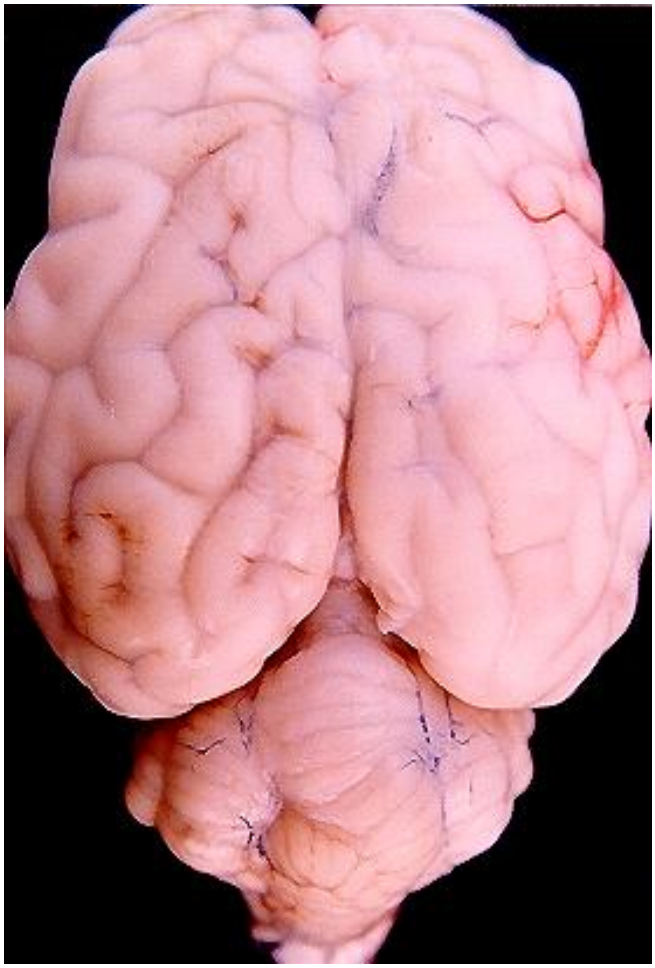

Fig 15. Improperly stained brain due to inadequate irrigation.

The basics of preparation of the specimen, tissue dissection, injection of the dyes and finally evaluating the specimen was a tedious process, during which many valuable lessons were learnt which helped us to improve upon the technique while dissecting upon subsequent specimens.

In the very first specimen on whom we had worked upon, it was very difficult to even dissect out the blood vessels at the base of the neck; which is the very first step of the preparation of the specimen. With each subsequent specimen we progressed to the next step of the exercise till the entire operation could be completed with finesse. The most trying moments were while inspecting a brain which had been prepared for 3 to 4 days, turn out to be inadequately stained (Fig 15). Essentially, *adequate irrigation of the blood vessels was the key to a well stained specimen*.

Poster colours and acrylic paints

Once reasonable confidence had been gained in the injection methodology, the rest of the time was spent in experimenting with different colouring agents in various concentrations, before injecting them into the blood vessels. Finally, both the arteries and veins were getting stained reasonably well by a mixture of paint (poster colour & acrylic paint) and water in a 1:1 ratio.

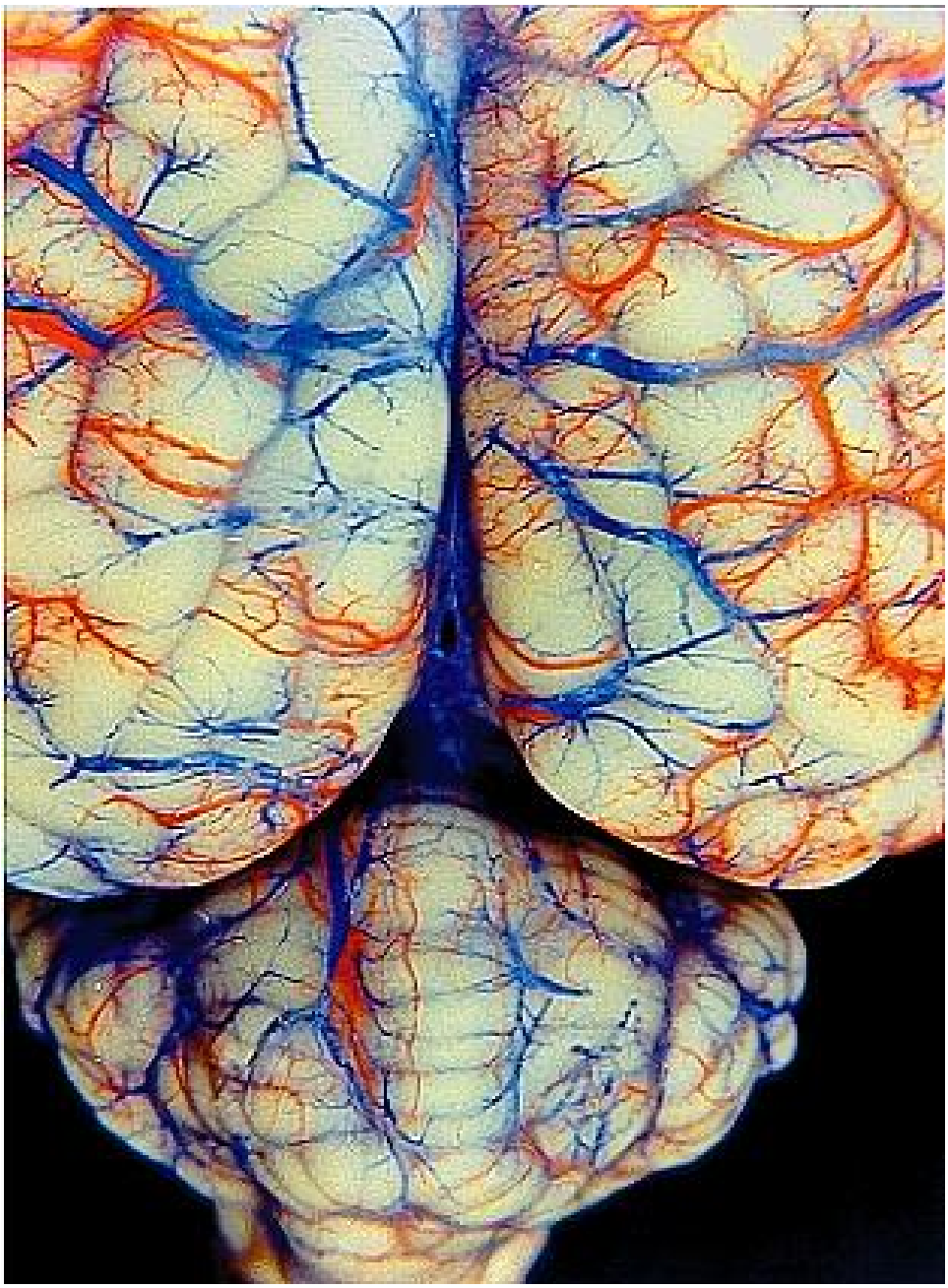

Fig 16. Poster colours – uniform staining

There were several **advantages** of using poster colours to stain the cerebral blood vessels.

- The staining was uniform; the entire length of the blood vessels appeared stained. (Fig. 16)
- The smaller, distal vessels were well stained.
- There was no leakage of the paint from the vessels, hence there was no smudging of the surrounding tissues as noticed in specimens stained with acrylic paints.

There were a few **disadvantages** noticed in specimens stained with poster colours:

- For a good cadaveric preparation, it is imperative that the blood vessels appear as if filled with blood. Poster colours, although, able to stain the blood vessels satisfactorily, did not cast them. Thus these blood vessels had a compressed appearance - as exemplified by the flattened left posterior communicating artery (arrow) in figure 17.
- The colours start fading after a week to ten days after the injection.

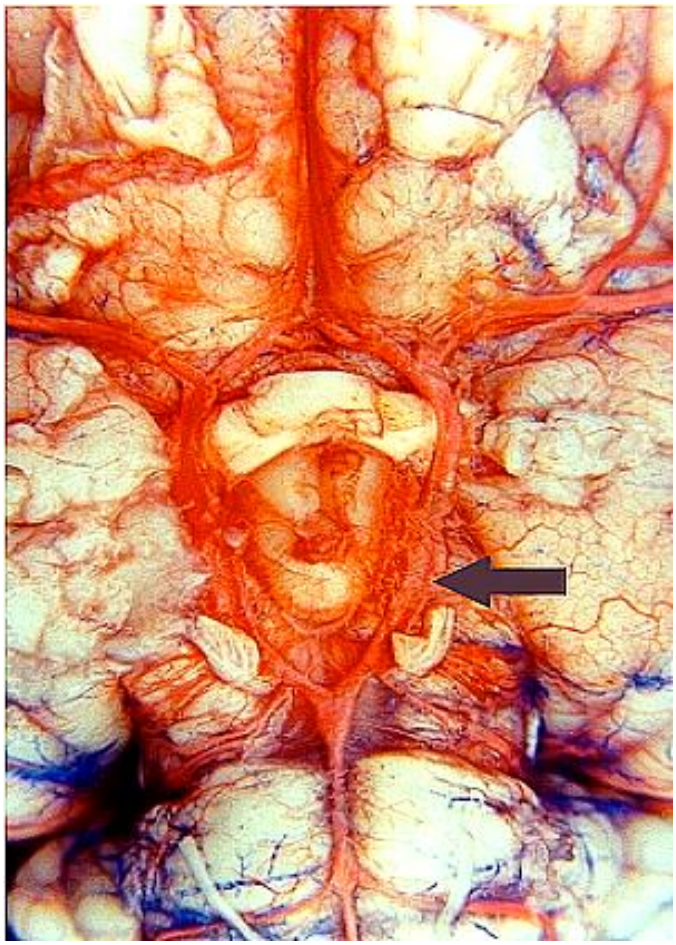

Fig 17. Poster colours do not cast blood vessels well. Arrow on the stained, but flattened left posterior communicating artery

Acrylic paints had the **advantage** of not fading with time, a quality which the poster colours did not possess. However, the following **disadvantages** were observed:

- Patchy uptake of colours in the blood vessels.
- The acrylic paints were more viscous than poster colours, hence the smaller arterioles and venules were not well stained.
- Mild leakage of paint from the vessels leading to smudging of the surrounding tissues and the dura mater.

Paints and suspending media

In an attempt to overcome the above difficulties it was decided to premix the paints with another substance (suspending medium), prior to the vascular injection. The following qualities were required of an ideal suspending medium:

- It should be soluble with the paints.
- It should have the optimum concentration to allow it to be injected into the blood vessels in its mixed state.
- The substance should not dilute the paints as the colour saturation would decrease.
- The substance must be in a fluid state initially and should harden after being injected into the blood vessel, thus casting it.
- It should not damage the surrounding tissues.
- It should delay the fading of colours with time, thus enabling us to store the brains for prolonged periods and to study them later.
- The colours must not leak out of the vessel if the brain is cut. This would allow us to prepare sections for histopathological studies.
- The prepared specimen was to be stored in 10% formalin. The nature of the substance must be such that its properties do not change when in contact with formalin.

Initially, we had considered experimenting with mixing the poster colours with epoxy resin, gum acacia and gelatin. However, literature is replete with instances where these substances have been mixed with paints for vascular injections, hence this idea was shelved.

The next step was to premix paint with other suspending media with which it was hoped that the blood vessels would get casted. Egg yolk and acrylic paint solution in a ratio of 2:1 was solidifying well within infant feeding tubes at 3 days and hence was injected into the internal jugular veins.

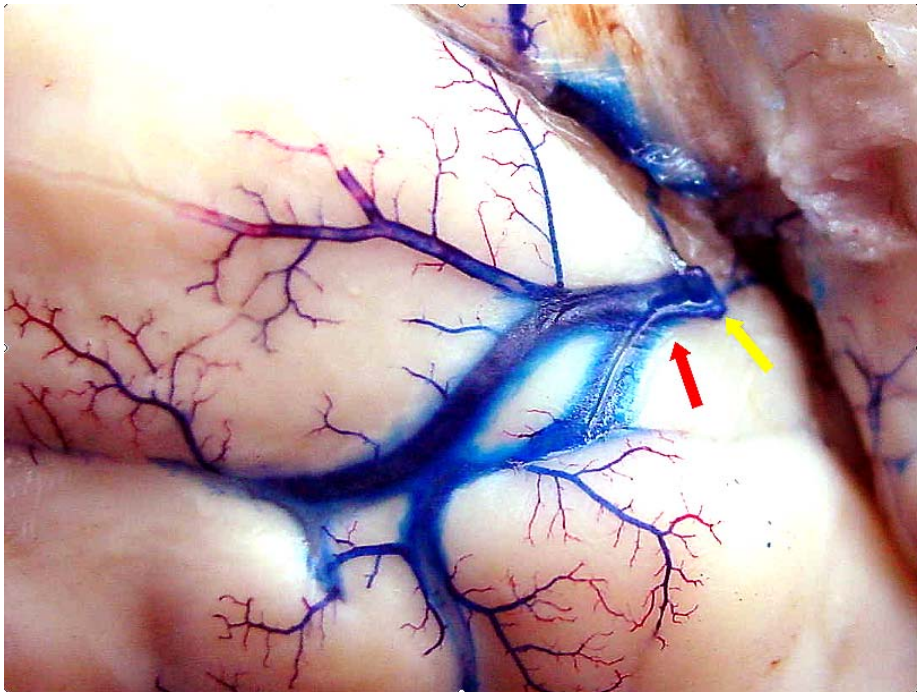

Fig 18. Veins stained with egg yolk and acrylic paint (2:1). There was partial filling up of the blood vessel with the dye (red arrow) and no spillage of the dye was observed (yellow arrow)

The cerebral veins thus stained showed partial filling up of the blood vessel (red arrow, fig 18). There was no spillage of the dye out of the vessel if it was cut (yellow arrow, fig 18). This was a definite step forward in the direction of making the staining agent solidify within blood vessels.

The egg yolk and acrylic paint solution was casting the blood vessels to a certain degree, but it did not compare with the standard staining techniques used for cadaver dissection.

Agar – acrylic paint mixture

After having experimented with various combinations and concentrations of stains and suspending media, it was concluded that the optimal staining and casting was obtained by injecting 2% agar – acrylic paint solution into the cerebral blood vessels. The acrylic paints provided colour to the vessels and mixed well with the agar solution, solidifying at 32 – 40 °C, rendering the blood vessels three dimensional (Figures 19, 20). This appearance of the cerebral blood vessels was seen not only on the cerebral convexities and circle of Willis but even in areas where blood vessels crossed sulci and were suspended unsupported over fissures. On cutting the blood vessels, the mixture did not leak out but remained embedded firmly within the vessels.

Fig 19. This photograph shows the left half of the Circle of Willis; the bifurcation of the ICA, the anterior and middle cerebral arteries. The arteries have been injected with a 2% agar-acrylic paint solution to stain them optimally.

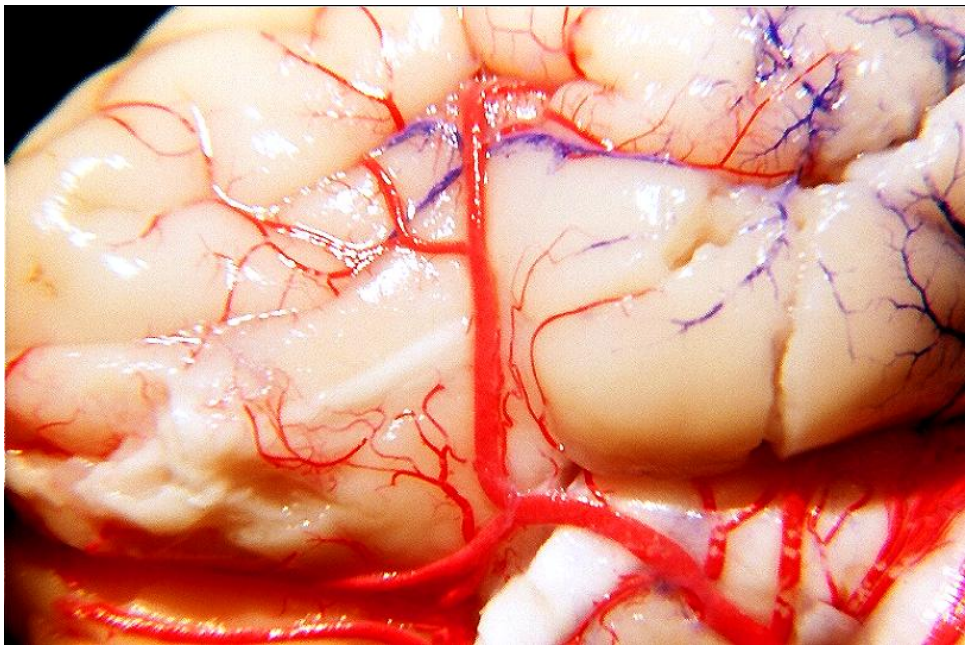

Fig 20. 2% agar-acrylic paint solution. Side view of the brain showing the ICA bifurcation and the course of the middle cerebral artery till it enters the Sylvian fissure.

The findings which were observed initially after craniectomy and dissecting out the brain were the same as storing the brain in formalin after a few weeks. The results obtained by staining the cerebral blood vessels with agar – acrylic paint solution were comparable to the staining of the blood vessels when injected with coloured silicones.

These properties of the staining solution make it a valuable tool in demonstrating the cerebral vasculature for gross anatomy and histopathological studies where the specimen would be required to be stored for a longer time period.

The total cost involved to stain one goat head with 2% agar – acrylic paint solution was Rs. 173.76 (3.70 USD). All the consumables were purchased locally. In contrast, the expenditure to stain one human cadaveric head with coloured silicone injection, as described earlier,[*] is approximately Rs 596.40 (12.71 USD).[†] Procuring the silicone would have involved cost of transportation to Vellore, in addition its cost.

Injection of the great vessels with agar- acrylic paint solution is not a simple process. The procedure involved is extremely messy and it requires a certain artistry to obtain good results. There are always

[*] Refer to table 1 on page 17

[†] There is no evidence in medical literature in which the cerebral blood vessels of a goat have been stained with coloured silicones. Hence, the cost involved to stain the human cerebral vessels with coloured silicones is mentioned, for comparison.

disappointments in terms of results with the specimens. Failure to obtain a good result is multifactorial; typically, why an injection failed cannot always be determined. However, hard work is certainly worthwhile because well injected specimens are critical for meaningful, educational dissection experiences.

CONCLUSIONS

This study was conducted to standardise a suitable and inexpensive dye to stain the cerebral blood vessels.

The agent which stained the cerebral blood vessels the best was poster colour, however when injected without the agar mixture, the blood vessels appeared flattened. Since the poster colours did not mix well with agar, they could not be used to stain or cast the blood vessels. Acrylic paints mixed well with agar. Although, these did not stain the blood vessels as vividly as the poster colours did, they were preferred because the vessels appeared three dimensional and the colours did not fade with time.

Egg yolk and acrylic paints cast the blood vessels to a certain degree, but were not satisfactory for anatomic study or to study surgical approaches.

After experimenting with several qualities of paints and paint-medium combinations, the dye which was staining cerebral blood vessels optimally was a 2% agar – acrylic paint mixture. The cost involved for staining one goat head with agar – acrylic paint solution was Rs. 173.76 (3.76 USD). All the consumables were purchased locally.

FUTURE DIRECTIONS

- To standardise this staining technique using agar-acrylic paint mixture in human cadaveric heads in optimum conditions.

- Specimens can be used for gross anatomical and histopathological study, since the dye hardens well within the vessels.

- Development of a cadaveric brain surgical skills laboratory - to study the anatomy and to practice surgical approaches and techniques on stained cadaver brains.

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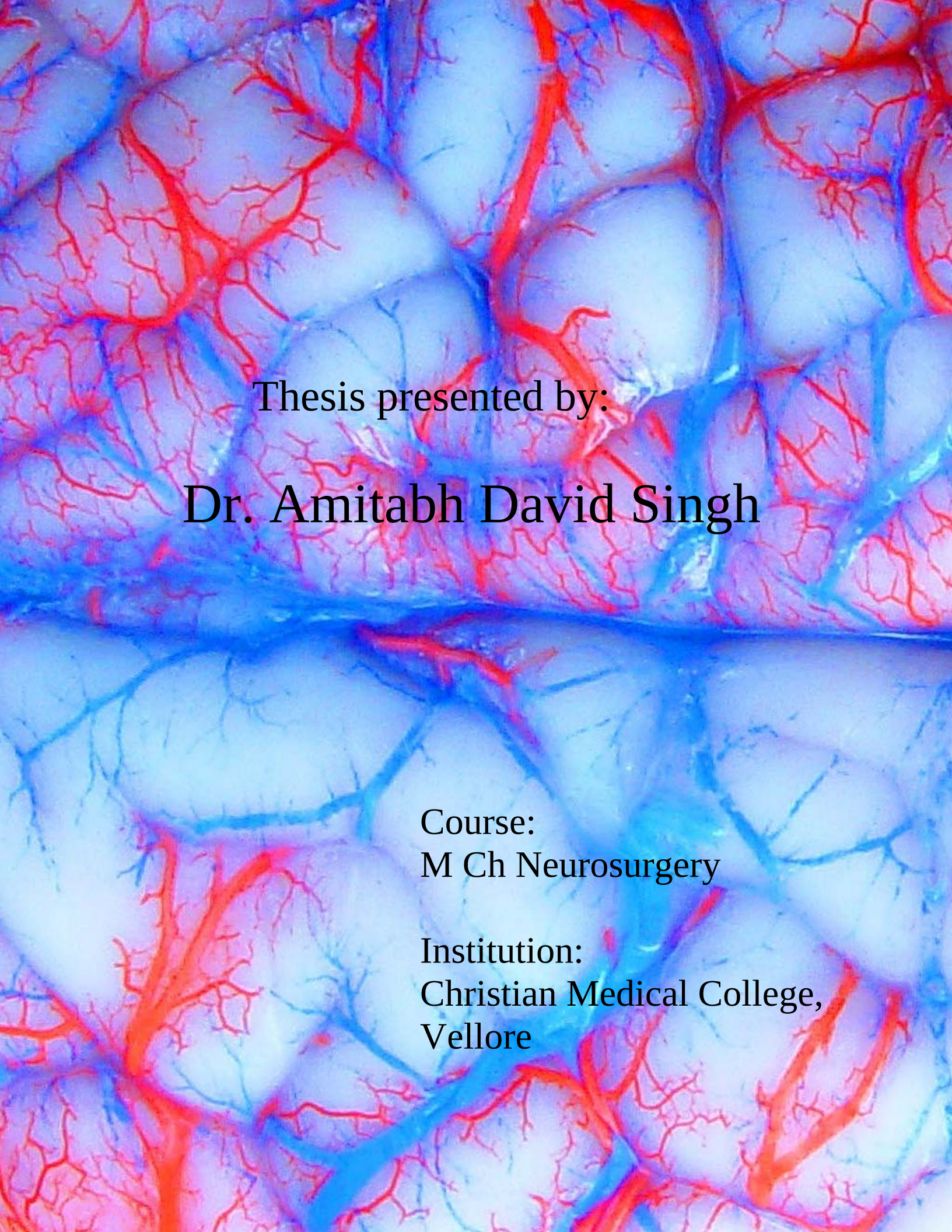

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